J. Pharm. Pharmacol. 1981, 33: 549–550
Communicated April 27, 1981

0022–3573/81/080549–02 $02.50/0

The metabolism and excretion in man of *NN*-dimethylamino-isopropanol and *p*-acetamido-benzoic acid after administration of isoprinosine

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A combination of *p*-acetamidobenzoate (PAcBA) salt of *NN*-dimethylamino-isopropanol (DIP) and inosine in a 3:1 molar ratio has been reported to have antiviral activity in tissue cultures and in animals (Gordon & Brown 1972; Muldoon et al 1972) and in man (Waldman et al 1977).

It has been used clinically at up to 4 g daily administered in divided doses 4–8 times a day (Waldman et al 1977). The combination (Isoprinosine, Inosiplex) is described as a 'complex' (Merck 1976) but what happens to this in vivo is not known and there are no reports of the metabolism of the product. Inosine cannot be measured but the present communication describes the metabolism of the other two components (DIP and PAcBA), their excretion and that of their metabolites after the 'complex' had been taken orally as a solution by two healthy fasting volunteers (aged 42 and 45 years). Urine was collected every half hour for the first 2 h and after that every hour up to 8 h. 16–20 urine samples were collected during the experiments which lasted 24 h.

Synthesis. DIP-*N*-oxide to be used as a reference sample was synthesized as described by Newport (personal communication). To 5 ml of DIP in 10 ml of ethanol was added 5 ml of 20% H_2O_2. The solution was left at room temperature for 24 h upon which solvents and unreacted amine were removed by vacuum distillation leaving the DIP-*N*-oxide as a white crystalline material the identity and purity of which were confirmed by n.m.r. and mass spectrometry as well as by t.l.c. (Table 1).

NN-Diethylamino-isopropanol (DIEP) to be used as internal standard for g.l.c. was synthesized as described by Mowry & Brode (1941). Its identity and purity were confirmed using n.m.r., mass spectrometry, and g.l.c.

The salt of *NN*-dimethylamino-isopropanol with *p*-acetamido benzoic acid (DIP-PAcBA) was a gift from Newport Pharmaceuticals International Inc.

Gas liquid chromatography was carried out on a Perkin Elmer F 30 with flame ionization detector. Column: 2 m × 4 mm (i.d.) glass packed with 28% Pennwalt + 4% KOH on Chromosorb R (Applied Science Laboratories). Temperatures were: oven 160 °C, injector 200 °C, detector 200 °C. Carrier gas: Nitrogen flow-rate 70 ml min^{-1}, hydrogen pressure 1·2 atm and air pressure 1·0 atm. Rt for DIP was 3·5 min and for DIEP 8·5 min.

High performance liquid chromatography was carried out on a Magnus Scientific apparatus equipped with a Milton Roy mini pump and a CE 212 variable wavelength monitor (CECIL instruments). Column: 250 mm × 4·6 mm stainless steel packed with ODS-Hypersil, particle size 5 μm (Magnus Scientific). Eluent: 0·05 M H_3PO_4 in water with 18% (v/v) of methanol. Flow rate 2·0 ml min^{-1}. Rt for PAcBA-glucuronide was 6 min, PAcBA 11 min, and succinylsulfathiazole (Internal standard) 12·5 min.

Thin layer chromatography. 20 × 20 cm plates coated with 0·25 mm layers of silica gel GF_{254} (Merck) were used. The solvent systems A–C and the R_F-values are given in Table 1. DIP and DIP-*N*-oxide were detected with Dragendorff's reagent.

Analysis

DIP. Urine (1·0 ml) and 0·1 ml of a 0·1% (v/v) solution of DIEP were pipetted into extraction tubes, cooled in an ice bath, and 1·0 g of solid KOH slowly dissolved by alternate vigorous shaking (cyclo-vortex mixer) and cooling in the ice bath. DIP was extracted with 100 μl of chloroform for 2 × 2 min by alternate vigorous vortexing and cooling, the extract centrifuged and 2 μl of the chloroform layer injected into the chromatograph. The extraction of DIP was almost quantitative. DIP-*N*-oxide did not interfere. Quantitation was by peak height and the concentration was determined in relation to a standard curve obtained from a set of standards diluted with blank urine and treated as the urine samples.

*DIP-*N*-oxide* required to be reduced quantitatively to the parent amine: To 2·5 ml of urine was added 100 μl 6 M HCl and 150 μl $TiCl_3$ (30% (w/v) in 24% HCl (w/v)). The reduction was complete in 30 min at room temperature. DIP-*N*-oxide was then determined by difference between DIP

Table 1. Thin layer chromatography of DIP and DIP-*N*-oxide.

Compound	R_F-values in solvent system		
	A	B	C
DIP	0·15	0·21	0·21
DIP-*N*-oxide	0·25	0·19	0·13

Solvent systems: A. n-butanol–acetic acid–water (4:1:2), B. chloroform–methanol (4:1), C. propanol-2–chloroform–conc. ammonium hydroxide (20:80:1).

* Recipient of a grant from 'The Danish Agricultural and Veterinary Research Council' no. 513–15165 and from Carlsbergfondet, Copenhagen, Denmark.

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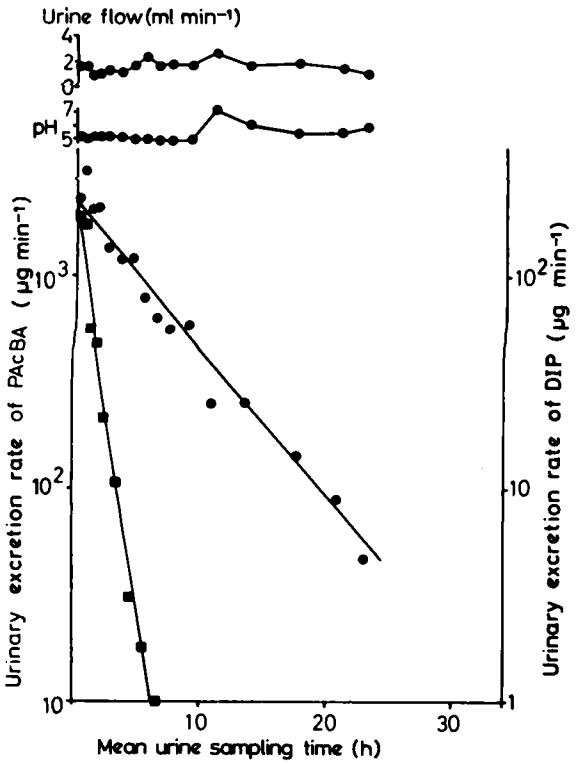

FIG. 1. Urinary excretion of DIP (●) and PAcBA (■) after administration of a solution of DIP-PAcBA to subject 1. Dose corresponding to 1·0 g of isoprinosine. Abscissa: Mean urine sampling time (h). Ordinate—left: Urinary excretion rate (μg min^{-1}) of PAcBA; right: Urinary excretion rate (μg min^{-1}) of DIP.

in reduced and unreduced samples. The identity of DIP-*N*-oxide in urine was confirmed by cochromatography with synthetic DIP-*N*-oxide.

PAcBA and PAcBA-glucuronide. The concentration of *p*-acetamido-benzoic acid (PAcBA) and its metabolite (PAcBA-glucuronide) was determined by h.p.l.c. To 1·0 ml of urine was added 0·1 ml of a 0·2% (w/v) of succinylsulfathiazole (internal standard). After mixing, 5 μl was injected and the absorbance at 260 nm recorded. PAcBA-glucuronide was identified by treatment of urine sample with β-glucuronidase. This led to the disappearance of the PAcBA-glucuronide peak and to a corresponding increase in the amount of PAcBA. The metabolite was unaffected by treatment with dilute acid but was labile when treated with dilute base releasing PAcBA (Newport personal communication 1980). This is characteristic for *O*-acyl glucuronides (Mandel 1971) and the metabolite may thus be an *O*-glucuronide which releases PAcBA on hydrolysis.

Results

Fig. 1 shows the results from subject I, the pattern was seen to be similar with subject II, the absorption and elimination of DIP was fast. The urinary excretion of unchanged DIP reached a maximum of 320 μg min^{-1} from 30 to 60 min after ingestion of the 'complex'. The elimination half-life was on average from the two subjects 3·5 h. 30% of DIP was recovered unchanged, and 65% as DIP-*N*-oxide giving a total recovery of DIP of 95% of the dose (Table 2).

Table 2. Recovery in man (% of initial dose) calculated to ∞ under acidic urine conditions of *NN*-dimethylamino-isopropanol (DIP) and its metabolite DIP-*N*-oxide and of *p*-acetamido-benzoic acid (PAcBA) and its metabolite PAcBA-glucuronide after administration of DIP-PAcBA in solution.

Compound	Subject	DIP	DIP-*N*-oxide	PAcBA	PAcBA-glucuronide
DIP-PAcBA (sol)	I	32	67	34	57
	II	30	64	31	51

The absorption and elimination of PAcBA was even faster than that of DIP (Fig. 1), and its urinary excretion reached a maximum of 1850 μg min^{-1} during the first 30 min. The elimination half-life was 50 min. 30% PAcBA was recovered unchanged and 55% as PAcBA-glucuronide. The total recovery of PAcBA was therefore 85% of the dose.

Discussion

The relative amount of DIP-*N*-oxide excreted in urine in the present experiments was always higher than that of the parent compound. Being an amino alcohol, DIP has a very high water solubility while DIP-*N*-oxide, as for other *N*-oxides (Testa & Jenner 1976), has an even higher solubility. Such high polarity means that tubular reabsorption does not occur and excludes the possibility of reduction of the *N*-oxide in the organism once it has been excreted in the urine. If dealkylation of DIP does take place, it may be a very minor metabolic pathway since no dealkylated metabolites could be detected in the urine samples.

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